



Review Article

Molecular classification of endometrial cancers: The way forward

Indranil Chakrabarti¹¹Dept. of Pathology, North Bengal Medical College, West Bengal, India

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ABSTRACT

Endometrial carcinomas are common gynecological malignancies which have traditionally been classified and graded on the basis of clinicopathological and histopathologic findings. However, these classifications are fraught with subjectivity and considerable overlaps. The new molecular basis of classification intends to provide more objective and accurate information even on diagnostic biopsy which can help the treating clinicians to decide upon the appropriate management at a very early stage. In this review, the need and evolution of the molecular classification will be discussed along with subsequent developments, as a step towards personalized medicine.

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1. Background

Cancer diagnostics and care have been revolutionized over the years with the advent of newer technologies and increasing understanding of tumor biology. Days are gone when the histomorphologic diagnosis based on light microscopy was the mainstay for treatment. Molecular diagnostics are becoming more and more pertinent for evolution of personalized medicine often known as precision medicine. This trend is seen in the case of endometrial carcinomas (EC) as well. Endometrial carcinomas are the fourth most common cancer in women in the developed world¹ and are on the rise. However, fortunately, in India, the incidence rates are low. Most of them present early and are associated with a favorable prognosis.²

In 1983, Bokhman first proposed the hypothesis of a dualistic model of endometrial cancers based on clinical, metabolic and endocrine features in which he described two distinctly different forms of endometrial carcinomas.^{3,4} He postulated that there are primarily two different pathogenetic types of EC—The Type I (frequency 65%) and Type II (frequency 35%). The Type I was thought to arise in women with obesity, hyperlipidemia

with signs of hyperestrogenism and was associated with features of hyperplasia of the stroma of the ovaries and endometrium. This group usually included well to moderately differentiated tumors, superficial invasion, favorable prognosis and good response to progestogens. The second pathogenetic type, which has no such signs, is characterized by poorly differentiated tumors, deeper invasion into the myometrium, higher rates of metastasis, decreased response to progestogens and doubtful prognosis.³

As per the World Health Organization (WHO) classification of tumors, there are several important histologic types of endometrial cancers which include the endometrioid type (with variants), serous carcinomas, clear cell carcinomas, carcinosarcomas etc. If we correlate these two classifications, the Type I hyperestrogenic tumors will include the endometrioid and mucinous types while serous carcinomas, clear cell carcinomas, undifferentiated carcinomas and carcinosarcomas will fall under the Type II category.

As more evidence emerged, it has now been hypothesized that type II cancers arise from a premalignant lesion – EIC (endometrial intraepithelial carcinoma), while the type I tumours are associated with a distinct premalignant condition – EIN (endometrial intraepithelial

E-mail address: drinch@rediffmail.com (I. Chakrabarti).

neoplasia).^{4–6}

However, there are some inherent problems of the Bokhman classification of ECs. First of all, the dualistic model is too simplistic and there appears considerable overlap of original symptoms and associations between the 2 types. Secondly, the original Bokhman classification did not include the clear cell, undifferentiated and carcinosarcomas, which, with time has been included under the Type II category. Moreover, though they belong to the two ends of the spectrum, some serous carcinomas (Type II) may behave like a Grade I endometrioid carcinoma (Type I) while some high grade endometrioid carcinomas may show clinical and pathological characteristics of serous carcinomas.⁴

The risk stratification of ECs depends on several factors including age, histological subtype, grade, disease extension, lymphovascular space invasion (LVSI), lymph node status etc but there has been reports of significant level of subjectivity and disagreement between pathologists as well as between diagnostic endometrial specimens and final hysterectomy specimens, in assigning the histological types and grade⁷ and even extent of cervical involvement.⁸ In certain cases, vascular pseudoinvasion also has been reported to cause significant problem in the assessment of LVSI particularly in laparoscopic hysterectomies.⁹ Bendifallah et al¹⁰ in their study on 5 major risk stratification systems (RSS) in EC inferred that none was highly accurate at stratifying risk of recurrence or metastases of early stage EC.

The situation is further complicated by the fact that even the same grade and histologic type of tumors have been found to show significant diversity in clinical outcomes.⁷

All these have posed serious doubts on the utility of the present classifications solely based on clinical associations and/or histological assessments and a need was generated to obtain more accurate, consistent and informative data which can help in planning an optimal treatment protocol for the individual patient.

2. The molecular classification

Molecular studies of ECs have shown to harbor various mutations like *PTEN*, *KRAS*, *CTNNB1*, *PIK3CA* and microsatellite instability (MSI) with loss of function of tumor suppressor gene *PTEN* being a major driver of endometrioid carcinomas and loss of p53 function being responsible for serous carcinomas.^{4,11}

However, it was in 2013 that the most large-scale molecular study on ECs was published by TCGA (The Cancer Genome Atlas Research Network). In this comprehensive study, the researchers characterized a group of 373 endometrial carcinomas, in which an integrated genomic, transcriptomic and proteomic characterization was done using array- and sequencing-based technologies.^{4,12} The network performed a

multiplatform analysis which included combination of next generation sequencing of whole genome, exome sequencing, copy number analysis and assessment of microsatellite instability (MSI) and based on the genomic data, proposed a new molecular classification of endometrial carcinomas.

Their results classified the endometrial carcinomas into 4 categories

1. *Polymerase ε (POLE)* ultramutated
2. Microsatellite instability hypermutated
3. Copy number – high
4. Copy number – low

The ultramutated *POLE* subgroup is characterised by an extremely high mutation rate (232×10^{-6} mutation/Mb) in conjunction with somatic exonuclease domain mutations (EDM) of the *POLE* gene which encodes the central catalytic and proofreading subunit of *Pole* (Polymerase Epsilon) DNA polymerase enzyme complex.^{4,11} This enzyme complex is responsible for leading strand DNA replication and also involved in the DNA repair correcting the possible errors that may occur during synthesis of DNA.^{1,4} This subgroup also harbor a high percent of C > A transversions, a low percent of C > G transversions, low copy number and more than 500 SNVs (single-nucleotide variants).¹ The network discovered 190 SMGs (significantly mutated genes) among which *PTEN* alteration was detected in 94.1% of tumours.⁴ The TCGA assessed the *POLE* status of the ECs by whole genome or exome sequencing but other researchers have used Sanger sequencing, gene panels, digital PCR or functional assays with equivalent results. This subgroup in itself is a novel finding, as the tumors harboring this mutation were associated with favorable prognosis with longer period of progress free survival even within high grade tumors. Histologically, in the TCGA study, the *POLE* ultramutated tumors included the endometrioid carcinomas ranging from low to mostly high grade types while none of the serous or mixed ECs were included in this subgroup.⁴ Further studies have shown that these *POLE*- ultramutated ECs are typically high-grade endometrioid ECs with a superficial broad front pattern of invasion with presence of tumor giant cells and prominent tumor-infiltrating lymphocytes or TILs.^{7,13–16}

In the TCGA, the tumors were tested for microsatellite instability (MSI) by a panel of 7 markers (4 mononucleotide repeat loci, 3 dinucleotide repeat loci) in addition to the recommended markers from the National Cancer Institute¹⁷ and classified into microsatellite- stable (MSS), low level MSI (MSI-L) and MSI (MSI-H).¹ The MSI (hypermuted) subgroup was thus formed by tumors characterized by a high mutation rate (18×10^{-6} mutations/Mb), low level of copy number alterations and MSI with frequently reduced *MLH1* gene expression due to hypermethylation of its promoter.^{4,11} 28.6% of low-grade

and 54.3% of high-grade endometrioid carcinomas were included in this subgroup in the TCGA study while none of the serous or mixed carcinomas were seen.^{4,12} PTEN mutations were found to be the most prevalent in this hypermutated/MSI+ subgroup.⁴

In this study, copy number analysis was done using Affymetrix SNP 6.0 microarrays using DNA originating from frozen tissue.¹ The copy number – high (serous-like) subgroup included the vast majority of serous carcinomas (97.7%), 19.6% of high-grade endometrioid ECs, 5% of low-grade endometrioid ECs and 75% of mixed carcinomas. These tumors were characterized by extensive copy number aberrations and a comparatively low mutation rate (2.3×10^{-6} mutations/Mb). The study detected eight SMGs in this group with TP53 being the most common mutated gene (91.7%). Other important mutations were also detected but PTEN mutations were infrequent in this subgroup.

The copy number low subgroup included those tumors which could not be placed in the above 3 subgroups. They mostly included the microsatellite- stable (MSS) endometrioid ECs and were characterized by low mutation rate (2.9×10^{-6} mutations/Mb). Histologically, 60% of low-grade ECs, 8.7% of high-grade ECs, 25% of mixed and 2.3% of serous carcinomas were also seen to belong to this subgroup. The study identified 16 SMGs in this subgroup along with increased expression of the progesterone receptors (PR) which indicates hormonal responsiveness of these tumours.⁴

Overall across the four TCGA subgroups, PTEN mutations were involved in 90% of all ECs while PIK3 pathway alterations were also common. KRAS mutations were much less frequent in tumors with copy number alterations (high/low) compared to the MSI+/hypermutated type.

The molecular classification generated tremendous positive response as they correlated very well with the clinical outcomes with survival rates being the best in POLE mutated tumors, followed by copy number-low, microsatellite instability and copy number-high carcinomas. Though this TCGA classification could benefit the patient risk stratification and optimal management, its wide clinical application was soon perceived to be difficult due to complexity and high expense of the used procedures.

Faced with the challenge, 2 teams of researchers developed some more easily applicable and affordable methodologies to evaluate molecular features of ECs. Though strictly speaking these methodologies, using formalin fixed, paraffin embedded (FFPE) tumoral tissue, do not recapitulate the same TCGA subgroups but they definitely identify prognostically distinct molecular subgroups.

Stelloo et al.^{18,19} determined the p53 status of tumors by using a combination of TP53 mutational testing and p53 immunohistochemistry (IHC) as a surrogate for the

copy number high subgroup since TP53 mutations are the most common mutation in this subgroup. However, it must be remembered that TP53 mutations are not specific for CN high tumors and can also be seen in the MSI Hypermutated and POLE ultramutated tumors.

The promega MSI analysis system and IHC for mismatch repair (MMR) proteins (MLH1, MSH2, MSH6, and PMS2) were performed to detect MSI while the POLE EDM hotspot mutations were detected by Sanger sequencing of Exon 9 and 13.¹

With these techniques, the research team initially assessed ECs with known high risk features from the PORTEC3 trial (n =116) They also analyzed DNA for hotspot mutations in 13 additional genes (BRAF, CDKNA2, CTNNB1, FBXW7, FGFR2, FGFR3, FOXL2, HRAS, KRAS, NRAS, PIK3CA, PPP2R1A, and PTEN) and protein expression of ER, PR, PTEN and ARID1a. Finally, they correlated the tumors with the outcomes in the form of recurrence-free and overall survival as well as the rates of distant metastasis.¹⁸

Thus, they came up with four molecular subgroups:

1. Group 1 - p53 (mutation identified),
2. Group 2- MSI,
3. Group 3 –POLE (POLE EDM identified)
4. Group 4 –NSMP, a group with ‘no specific molecular profile’

Their findings showed that MSI tumors and POLE mutated tumors fared better in terms of survival outcomes and rates of metastasis compared to the p53 mutated or even the NSMP groups. This discrepancy with the TCGA study may be due to the high number of high grade tumors included in the cohort (86 endometrioid; 12 serous; and 18 clear cell carcinomas).

The Leiden/TransPORTEC group then applied the molecular tests to a larger cohort early-stage endometrioid ECs of high- intermediate risk from the two randomized trials (PORTEC-1 and -2) and obtained outcomes which matched more closely with the TCGA group.^{1,19} MSI, p53, L1CAM and CTNNB1 also proved to be independent prognosticators in this analysis.¹⁹

Talhok et al developed a sequence of testing and methods known as Proactive Molecular Risk classification tool for Endometrial cancers (ProMisE) using the molecular techniques for identifying exonuclease domain mutations (EDMs) in POLE and immunohistochemistry for mismatch repair (MMR) proteins and p53. Using these tests in several cohorts they could identify four molecular subgroups:

1. MMR-D (D-deficient)
2. POLE EDM
3. p53wt (wild type)
4. p53abn (abnormal).^{1,20}

The proposed molecular decision tree suggests performance of IHC first for the detection of ‘MMRD’ (deficient)

subgroup by testing for two mismatch repair (MMR) proteins: MSH6 and PMS2. Cases which are having intact MMR are then sequenced using digital PCR to identify POLE EDMs. Those with wild type POLE are then tested for p53 wild type ‘p53wt’ or p53 abnormal (null/missense mutations) ‘p53abn’

Very interestingly, they have also demonstrated that certain clinicopathological features are consistently present in some subgroups. For example, the patients of p53 abn subgroup are usually older and thinner with the highest proportion of high grade, advanced stage, non-endometrioid types of ECs. Again, the women whose EC harbor POLE EDMs commonly are younger in age and in spite of aggressive pathologic features like grade 3 tumors deep myometrial invasion and LVSI, usually have favorable outcomes. However, in spite of similar ‘uterine factors’ to the POLE subgroup, the MMR-D tumors have poor prognosis only better than the p53 abn subgroup.¹

The risk stratification efficacy of both Leiden/TransPORTEC group and ProMisE improved when the clinicopathological features were integrated with the molecular features.

More encouragingly, both the groups demonstrated high concordance between molecular classification in diagnostic (curetage) and final hysterectomy samples far exceeding any histologic or clinicopathological classification.

Thus, though our knowledge of the molecular biology of ECs is still evolving, the molecular fingerprinting of these tumors by more affordable techniques definitely paves the way for objective categorization of endometrial carcinomas, better risk stratification and unequivocal help towards decision of appropriate management.

3. Source of Funding

None.

4. Conflict of Interest

None.

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Author biography

Indranil Chakrabarti Associate Professor

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